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| (54) Title: A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING LUNG CANCER (57) Abstract The present invention provides a new method for detecting, diagnosing, monitoring, staging, and prognosticating lung cancer. | | |

**A NOVEL METHOD OF DIAGNOSING,
MONITORING, AND STAGING LUNG CANCER**

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, and prognosticating cancers, particularly lung cancer.

BACKGROUND OF THE INVENTION

Primary lung cancer is divided into three main types including small cell lung cancer, non-small cell lung cancer, and mesothelioma. Small cell lung cancer is also called "Oat Cell" lung cancer because the cancer cells are a distinctive oat shape. There are three types of non-small cell lung cancer which are grouped together based upon similar behavior patterns and response to treatment which is different from small cell lung cancer. The three types of non-small cell lung cancer are squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Squamous cell cancer is the most common type of lung cancer. It develops from the cells that line the airways. Adenocarcinoma also develops from the cells that line the airways, but it develops from a particular type of cell that produces mucus (phlegm). In large cell lung cancer, the cells appear large and rounded when viewed under a microscope. Mesothelioma is a rare type of cancer which affects the covering of the lung, the pleura. It is often caused by exposure to asbestos.

Secondary lung cancer is cancer that has started somewhere else in the body (for example, the breast or bowel) and spread to the lungs. The choice of treatment depends on where the cancer began. For example, cancer that has spread from the breast should respond to breast cancer treatments and cancer that has spread from the bowel should respond to bowel

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cancer treatments. The stage of a cancer provides information regarding how far a cancer has spread. Staging is important because treatment of the cancer is often decided based upon its stage. Staging is different for non-small cell versus small cell cancers of the lung.

Non-small cell cancer is divided into four stages. Stage I is very localized cancer with no cancer in the lymph nodes. In stage II, cancer has spread to the lymph nodes at the top of the affected lung. In stage III, cancer has spread near to where the cancer started. This can be to the chest wall, the covering of the lung (pleura), the middle of the chest (mediastinum) or other lymph nodes. Stage IV cancer has spread to another part of the body.

Small cell lung cancers are divided into two groups. This is because small cell lung cancer often spreads quite early. Even if spreading of the cancer is not visible on scans, it is likely that some cancer cells will have broken away and traveled through the bloodstream or lymph system. Accordingly, it is often preferred to treat small cell lung cancers as if they have spread, whether or not any secondary cancer is seen.

The two stages of small cell lung cancers are limited disease, that is cancer that can only be seen in one lung and in nearby lymph nodes, and extensive disease, that is cancer that has spread outside the lung to the chest or to other parts of the body. Because surgery is not usually used to treat small cell cancer, except in very early cases, the staging is not as important as it is with some other types of cancer. Chemotherapy with or without radiotherapy is usually preferred for treatment of small cell lung cancers. Initial scans and tests are used for comparison with later scans and test to see how well a patient is responding to treatment.

Procedures used for detecting, diagnosing, monitoring, staging and prognosticating lung cancer are of critical importance to the outcome of the patient. For example,

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patients diagnosed with early lung cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized lung cancer. New diagnostic methods which are more sensitive and specific for detecting early lung cancer are clearly needed.

Lung cancer patients are also closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease of metastasis. There is clearly a need for a lung cancer marker which is more sensitive and specific in detecting lung cancer recurrence.

Another important step in managing lung cancer is determination of the stage of the disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Generally, pathological staging of lung cancer is preferable over clinical staging because the former gives a more accurate prognosis. However, clinical staging would be preferred were it at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of lung cancer would be improved by detecting new markers in cells, tissues or bodily fluids which could differentiate between different stages of invasion.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging and prognosticating lung cancer via six (6) Lung Specific Genes (LSGs). The six LSGs refer, among other things, to native proteins expressed by the genes comprising the polynucleotide sequences of any of SEQ ID NO: 1, 2, 3, 4, 5 or 6. In the alternative, what is meant by the six LSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5 or 6 or

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levels of the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5 or 6.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of lung cancer in a patient which comprises measuring levels of LSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of LSG with levels of LSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in the measured LSG levels in the patient versus levels of LSG in the control is associated with lung cancer.

Another object of the present invention is to provide a method of diagnosing metastatic lung cancer in a patient which comprises measuring LSG levels in a sample of cells, tissue, or bodily fluid from the patient and comparing the measured LSG levels with levels of LSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured LSG levels in the patient versus levels of LSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging lung cancer in a patient which comprises identifying a patient having lung cancer, measuring levels of

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LSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured LSG levels with levels of LSG in preferably the same cells, tissue or bodily fluid type of a control. An increase in measured LSG levels in the patient versus LSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of LSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

Another object of the present invention is to provide a method of monitoring lung cancer in a patient for the onset of metastasis. The method comprises identifying a patient having lung cancer that is not known to have metastasized, periodically measuring levels of LSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured LSG levels with levels of LSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured LSG levels versus control LSG levels is associated with a cancer which has metastasized.

Yet another object of the present invention is to provide a method of monitoring the change in stage of lung cancer in a patient which comprises identifying a patient having lung cancer, periodically measuring levels of LSG in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured LSG levels with levels of LSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured LSG levels versus the control LSG levels is associated with a cancer which is progressing and a decrease in the measured LSG levels versus the control LSG levels is associated with a cancer which is regressing or in remission.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be

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understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of LSG with those of LSG in a normal human control. What is meant by "levels of LSG" as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4, 5, or 6. In the alternative, what is meant by "levels of LSG" as used herein, means levels of the native mRNA encoded by the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, or 6 or levels of the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, or 6. Such levels are preferably measured in at least one of, cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing over-expression of LSG protein compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including lung cancer. Any of the six LSGs may be measured alone in the methods of the invention, or all together or any combination of the six.

By "control" it is meant a human patient without cancer and/or non cancerous samples from the patient, also referred to herein as a normal human control; in the methods for diagnosing or monitoring for metastasis, control may also

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include samples from a human patient that is determined by reliable methods to have lung cancer which has not metastasized.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as LSG. Other cancer markers, in addition to LSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of lung cancer by analyzing for changes in levels of LSG in cells, tissues or bodily fluids compared with levels of LSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of LSG in the patient versus the normal human control is associated with the presence of lung cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as LSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic lung cancer in a patient having lung cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having lung cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art. For example, in the case of lung cancer, patients are typically diagnosed with lung cancer following traditional detection methods.

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In the present invention, determining the presence of LSG level in cells, tissues, or bodily fluid, is particularly useful for discriminating between lung cancer which has not metastasized and lung cancer which has metastasized. Existing techniques have difficulty discriminating between lung cancer which has metastasized and lung cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues, or bodily fluid is LSG, and are compared with levels of LSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just LSG in serum, this level is preferably compared with the level of LSG in serum of a normal human patient. An increase in the LSG in the patient versus the normal human control is associated with lung cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as LSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

Staging

The invention also provides a method of staging lung cancer in a human patient.

The method comprises identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for LSG. Then, the method compares LSG levels in such cells, tissues, or bodily fluid with levels of LSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in LSG levels in the patient versus the normal human control is.

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associated with a cancer which is progressing and a decrease in the levels of LSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring lung cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for LSG; comparing the LSG levels in such cells, tissue, or bodily fluid with levels of LSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in LSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this inventions is a method of monitoring the change in stage of lung cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for LSG; comparing the LSG levels in such cells, tissue, or bodily fluid with levels of LSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in LSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of LSG is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression, such as LSG of the present invention, in

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a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, in situ hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to LSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to LSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to LSG is incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time LSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to LSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to LSG. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to LSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of LSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

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A competition assay may be employed wherein antibodies specific to LSG attached to a solid support and labeled LSG and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of LSG in the sample.

Nucleic acid methods may be used to detect LSG mRNA as a marker for lung cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASBA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the LSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the LSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling

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of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Example 1: LSGs

Searches were carried out and LSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:

1. Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.
2. Subsetting is similar to library comparison but allows the identification of clones expressed in a pool of

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libraries and absent or expressed at a lower level in a second pool of libraries.

3. Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northern's to determine the tissue sources of their component ESTs.

4. Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins. Some examples in this database include Transcription Factors and Proteases. Some lead were identified by searching in this database for clones whose component EST's showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component EST's were exclusively or more frequently found in libraries from specific tumors. Individual candidate clones were examined in detail by checking where each EST originated.

TABLE 1: LSGs

| SEQ ID NO | Clone ID | Gene ID | |
|-----------|----------|---------|---------------------|
| 1 | 126758 | 29997 | Library Comparisons |
| 2 | 2798946 | 26723 | Library Comparisons |
| 3 | 3107312 | 242842 | Transcript Imaging |
| 4 | 1472038 | 51968 | Transcript Imaging |
| 5 | 126263 | 221807 | Transcript Imaging |
| 6 | 586271 | 242745 | Transcript Imaging |

The following example was carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A

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LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 2: Relative Quantitation of Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene was evaluated for every example in normal and cancer tissue. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

Comparative Examples

For comparative examples similar mRNA expression analysis for genes coding for the diagnostic markers PSA (Prostate Specific Antigen) and PLA2 (Phospholipase A2) was performed. PSA is the only cancer screening marker available in clinical laboratories. When the panel of normal pooled tissues was analyzed, PSA was expressed at very high levels in prostate, with a very low expression in breast and testis. After we analyzed more than 55 matching samples from 14 different tissues, the data corroborated the tissue specificity seen with normal tissue samples. We compared PSA expression in cancer and normal adjacent tissue for 12 matching samples of prostate tissue. The relative levels of PSA were higher in 10 cancer samples (83%). Clinical data recently obtained support the utilization of PLA2 as a staging marker for late stages of prostate cancer. Our mRNA expression data showed overexpression of the mRNA in 8 out of the 12 prostate matching samples analyzed (66%). The tissue specificity for PLA2 was not as good as the one described for PSA. In addition to prostate, also small intestine, liver, and pancreas showed high levels of mRNA expression for PLA2.

Measurement of SEQ ID NO:1; Clone ID 126758; Gene ID 29997 (Lng101)

The absolute numbers as depicted in Table 2 are relative levels of expression of LSG Lng101 (SEQ ID NO:1) in 12 normal different tissues. All the values are compared to normal testis (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

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Table 2: Relative levels of Lng101 Expression in Pooled Samples

| Tissue | NORMAL |
|-----------------|---------------|
| Brain | 0 |
| Heart | 1.55 |
| Kidney | 0 |
| Liver | 0 |
| Lung | 72716 |
| Mammary Gland | 2 |
| Prostate | 0 |
| Small Intestine | 0 |
| Spleen | 0 |
| Testis | 1 |
| Thymus | 0 |
| Uterus | 0 |

The relative levels of expression in Table 2 show that mRNA expression of the LSG Lng101 (SEQ ID NO:1) is very high (72716) in lung compared with all the other normal tissues analyzed. Testis, the calibrator, with a relative expression level of 1, heart (1.55), and mammary gland (2) are the only tissues expressing the mRNA for Lng101. These results demonstrated that Lng101 mRNA expression is highly specific for lung.

The absolute numbers in Table 2 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 3.

The absolute numbers depicted in Table 3 are relative levels of expression of Lng101 in 44 pairs of matching samples. All the values are compared to normal testis (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

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Table 3: Relative Levels of Lng101 Expression in Individual Samples

| Sample ID | Cancer Type | Tissue | Cancer | Matching Normal |
|-----------|-----------------------------------|---------|--------|-----------------|
| Lng AC82 | Adenocarcinoma | Lung 1 | 17199 | 92042 |
| Lng 60XL | Adenocarcinoma | Lung 2 | 4603 | 49971 |
| Lng AC66 | Adenocarcinoma | Lung 3 | 7358 | 116907 |
| Lng AC69 | Adenocarcinoma | Lung 4 | 82953 | 47644 |
| Lng AC11 | Adenocarcinoma | Lung 5 | 37771 | 496008 |
| Lng AC39 | Adenocarcinoma | Lung 6 | 2487 | 15771 |
| Lng AC32 | Adenocarcinoma | Lung 7 | 12634 | 204254 |
| Lng SQ9X | Squamous cell carcinoma | Lung 8 | 90774 | 14462 |
| Lng SQ32 | Squamous cell carcinoma | Lung 9 | 6677 | 677567 |
| Lng SQ80 | Squamous cell carcinoma | Lung 10 | 50711 | 47151 |
| Lng SQ16 | Squamous cell carcinoma | Lung 11 | 396 | 41333 |
| Lng SQ79 | Squamous cell carcinoma | Lung 12 | 10261 | 354395 |
| Lng 47XQ | Squamous cell carcinoma | Lung 13 | 2513 | 5293 |
| Lng SQ44 | Squamous cell carcinoma | Lung 14 | 69033 | 72 |
| Lng 90X | Squamous cell carcinoma | Lung 15 | 678 | 14715 |
| Lng LC71 | Large cell carcinoma | Lung 16 | 155332 | 44762 |
| Lng LC109 | Large cell carcinoma | Lung 17 | 10191 | 322737 |
| Lng 75XC | Metastatic from bone cancer | Lung 18 | 222033 | 165291 |
| Lng MT67 | Metastatic from renal cell cancer | Lung 19 | 189 | 35982 |

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| | | | | |
|--------------|-----------------------------|---------------|------|------|
| Lng MT71 | Metastatic from melanoma | Lung 20 | 122 | 4270 |
| Bld 32XK | | Bladder 1 | 0 | 0 |
| Bld 46XK | | Bladder 2 | 0 | 0 |
| Clm AS45 | | Colon 1 | 0 | 0 |
| Clm C9XR | | Colon 2 | 0 | 0 |
| Cvx KS52 | | Cervix 1 | 0 | 0 |
| Cvx NK23 | | Cervix 2 | 0 | 0 |
| End 28XA | | Endometrium 1 | 0 | 0 |
| End 12XA | | Endometrium 2 | 0 | 0 |
| Kid 106XD | | Kidney 1 | 0 | 0 |
| Kid 107XD | | Kidney 2 | 0 | 0 |
| Liv 94XA | | Liver 1 | 0 | 0 |
| Liv 15XA | | Liver 2 | 0 | 0 |
| Mam 82XI | | Mammary 1 | 0 | 0 |
| Mam A06X | | Mammary 2 | 0 | 0 |
| Pan 71XL | | Pancreas 1 | 0 | 0 |
| Pan 77X | | Pancreas 2 | 0 | 0 |
| Pro 20XB | | Prostate 1 | 0 | 0 |
| Pro 12B | | Prostate 2 | 0 | 0 |
| SmI 21XA | | Sm. Int. 1 | 0 | 0 |
| SmI H89 | | Sm. Int. 2 | 0 | 0 |
| Sto AC44 | | Stomach | 13 | 0 |
| Tst 39X | | Testis | 4315 | 0 |
| Utr 135XO | | Uterus 1 | 0 | 0 |
| Utr 141XO | | Uterus 2 | 0 | 0 |

0= Negative

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In the analysis of matching samples, the higher levels of expression were in lung, showing a high degree of tissue specificity for this tissue. These results confirmed the tissue specificity results obtained with the panel of normal pooled samples (Table 2).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 3 shows overexpression of LSG Lng101 in 6 lung cancer tissues compared with their respective normal adjacent (lung samples #4, 8, 10, 14, 16, and 18). There was overexpression in the cancer tissue for 30% of the lung matching samples tested (total of 20 lung matching samples).

Altogether, the high level of tissue specificity, plus the mRNA overexpression in 30% of the lung matching samples tested are demonstrative of LSG Lng101 (SEQ ID NO:1) being a diagnostic marker for lung cancer. The amino acid sequence encoded by Lng101 (SEQ ID NO:1) is depicted in SEQ ID NO: 7.

Measurement of SEQ ID NO:3; Clone ID 3107312; Gene ID 242842 (Lng105)

The absolute numbers depicted in Table 4 are relative levels of expression of LSG Lng105 (SEQ ID NO:3) in 12 normal different tissues. All the values are compared to normal kidney (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

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Table 4: Relative levels of Lng105 Expression in Pooled Samples

| Tissue | NORMAL |
|-----------------|---------------|
| Brain | 1 |
| Heart | 1.11 |
| Kidney | 558 |
| Liver | 0 |
| Lung | 9248 |
| Mammary Gland | 6 |
| Muscle | 0 |
| Prostate | 0 |
| Small Intestine | 87 |
| Testis | 50 |
| Thymus | 6 |
| Uterus | 23 |

The relative levels of expression in Table 4 show that mRNA expression of LSG Lng105 (SEQ ID NO:3) is more than 16 fold higher in the pool of normal lung (9248) compared with the next higher expressor (558 for kidney). All the other pooled tissues samples analyzed showed a very low level of expression for Lng105 (SEQ ID NO:3). These results demonstrate that mRNA expression of LSG Lng105 (SEQ ID NO:3) is highly specific for lung.

The absolute numbers in Table 4 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 5.

The absolute numbers depicted in Table 5 are relative levels of expression of Lng105 (SEQ ID NO:3) in 61 pairs of matching samples. All the values are compared to normal small intestine (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

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Table 5: Relative Levels of Lng105 Expression in Individual Samples

| Sample ID | Cancer Type | Tissue | Cancer | Matching Normal |
|------------------|-------------------------|---------------|---------------|------------------------|
| Lng AC82 | Adenocarcinoma | Lung 1 | 1278 | 742 |
| Lng C17X | Adenocarcinoma | Lung 2 | 1272 | 1948 |
| Lng 60XL | Adenocarcinoma | Lung 3 | 4345 | 2188 |
| Lng AC66 | Adenocarcinoma | Lung 4 | 1531 | 1558 |
| Lng AC69 | Adenocarcinoma | Lung 5 | 7232 | 913 |
| Lng AC88 | Adenocarcinoma | Lung 6 | 7724 | 24749 |
| Lng AC11 | Adenocarcinoma | Lung 7 | 690 | 21545 |
| Lng AC39 | Adenocarcinoma | Lung 8 | 16904 | 370 |
| Lng AC90 | Adenocarcinoma | Lung 9 | 14614 | 34 |
| Lng AC32 | Adenocarcinoma | Lung 10 | 8720 | 5061 |
| Lng SQ9X | Squamous cell carcinoma | Lung 11 | 3603 | 659 |
| Lng SQ45 | Squamous cell carcinoma | Lung 12 | 32998 | 1333 |
| Lng SQ56 | Squamous cell carcinoma | Lung 13 | 829 | 15077 |
| Lng SQ14 | Squamous cell carcinoma | Lung 14 | 7 | 6865 |
| Lng SQ32 | Squamous cell carcinoma | Lung 15 | 976 | 10227 |
| Lng SQ80 | Squamous cell carcinoma | Lung 16 | 2769 | 3554 |
| Lng SQ16 | Squamous cell carcinoma | Lung 17 | 198 | 292 |
| Lng SQ79 | Squamous cell carcinoma | Lung 18 | 1128 | 7777 |
| Lng C20X | Squamous cell carcinoma | Lung 19 | 4 | 20 |
| Lng 47XQ | Squamous cell carcinoma | Lung 20 | 276 | 117 |

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| | | | | |
|-----------|-----------------------------------|---------------|--------|-------|
| Lng SQ44 | Squamous cell carcinoma | Lung 21 | 3126 | 1 |
| Lng BR94 | Squamous cell carcinoma | Lung 22 | 709 | 6 |
| Lng 90X | Squamous cell carcinoma | Lung 23 | 258 | 590 |
| Lng LC71 | Large cell carcinoma | Lung 24 | 155332 | 44762 |
| Lng LC109 | Large cell carcinoma | Lung 25 | 34280 | 33112 |
| Lng 75XC | Metastatic from bone cancer | Lung 26 | 749 | 902 |
| Lng MT67 | Metastatic from renal cell cancer | Lung 27 | 70 | 6985 |
| Lng MT71 | Metastatic from melanoma | Lung 28 | 742 | 15992 |
| Bld 32XK | | Bladder 1 | 1 | 0 |
| Bld 46XK | | Bladder 2 | 0 | 0 |
| Cvx KS52 | | Cervix 1 | 4 | 0 |
| Cvx NK23 | | Cervix 2 | 1 | 0 |
| Cln AS45 | | Colon 1 | 0 | 1 |
| Cln C9XR | | Colon 2 | 2 | 1 |
| Cln CM67 | | Colon 3 | 0 | 0 |
| End 28XA | | Endometrium 1 | 7 | 4 |
| End 12XA | | Endometrium 2 | 0 | 0 |
| Kid 106XD | | Kidney 1 | 0 | 186 |
| Kid 107XD | | Kidney 2 | 82 | 458 |
| Kid 109XD | | Kidney 3 | 169 | 438 |
| Kid 10XD | | Kidney 4 | 21 | 186 |
| Kid 11XD | | Kidney 5 | 586 | 110 |
| Liv 94XA | | Liver 1 | 1 | 0 |

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| | | | | |
|-----------|--|------------|----|---|
| Liv 15XA | | Liver 2 | 1 | 0 |
| Mam A06X | | Mammary 1 | 1 | 0 |
| Mam B011X | | Mammary 2 | 13 | 0 |
| Mam 12X | | Mammary 3 | 0 | 0 |
| Mam 59X | | Mammary 4 | 0 | 0 |
| Ovr 103X | | Ovary 1 | 15 | 2 |
| Pan 71XL | | Pancreas 1 | 1 | 0 |
| Pan 77X | | Pancreas 2 | 4 | 0 |
| Pro 20XB | | Prostate 1 | 1 | 1 |
| Pro 12B | | Prostate 2 | 8 | 0 |
| SmI 21XA | | Sm. Int. 1 | 4 | 0 |
| SmI H89 | | Sm. Int. 2 | 1 | 0 |
| Sto AC44 | | Stomach 1 | 0 | 2 |
| Sto AC99 | | Stomach 2 | 6 | 2 |
| Tst 39X | | Testis | 28 | 2 |
| Utr 85XU | | Uterus 1 | 3 | 2 |
| Utr 135XO | | Uterus 2 | 2 | 0 |
| Utr 141XO | | Uterus 3 | 2 | 6 |

0= Negative

In the analysis of matching samples, the higher levels of expression were in lung showing a high degree of tissue specificity for lung tissue. These results confirm the tissue specificity results obtained with normal pooled samples (Table 4).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the

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normal adjacent). Table 5 shows overexpression of LSG Lng105 (SEQ ID NO:3) in 13 lung cancer tissues compared with their respective normal adjacent (lung samples #1, 3, 5, 8, 9, 10, 11, 12, 20, 21, 22, 24, and 25). There is overexpression in the cancer tissue for 46% of the colon matching samples tested (total of 28 lung matching samples).

Altogether, the high level of tissue specificity, plus the mRNA overexpression in almost half of the lung matching samples tested are demonstrative of Lng105 (SEQ ID NO:3) being a diagnostic marker for lung cancer. The amino acid sequence encoded by Lng105 (SEQ ID NO:3) is depicted as SEQ ID NO:8.

Measurement of SEQ ID NO:6; Clone ID 586271; Gene ID 242745 (Lng107)

The absolute numbers depicted in Table 6 are relative levels of expression of LSG Lng107 (SEQ ID NO:6) in 12 normal different tissues. All the values are compared to normal mammary gland (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 6: Relative levels of Lng107 Expression in Pooled Samples

| Tissue | NORMAL |
|-----------------|---------------|
| Bladder | 0 |
| Heart | 0 |
| Kidney | 0 |
| Liver | 0 |
| Lung | 23 |
| Mammary Gland | 1 |
| Muscle | 0 |
| Prostate | 0 |
| Small Intestine | 0 |
| Testis | 0 |
| Thymus | 0 |
| Uterus | 0 |

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The relative levels of expression in Table 6 show that mRNA expression of LSG Lng107 (SEQ ID NO:6) is 23 fold higher in the pool of normal lung (23) compared to the expression level in the calibrator mammary gland (1). All the other tissues analyzed were negative for Lng107 (SEQ ID NO:6). These results demonstrate that Lng107 mRNA expression is highly specific for lung.

The absolute numbers in Table 6 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 7.

The absolute numbers depicted in Table 7 are relative levels of expression of LSG Lng107 (SEQ ID NO:6) in 57 pairs of matching samples. All the values are compared to normal prostate (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 7: Relative Levels of Lng107 Expression in Individual Samples

| Sample ID | Cancer Type | Tissue | Cancer | Matching Normal |
|-----------|----------------|---------|--------|-----------------|
| Lng AC82 | Adenocarcinoma | Lung 1 | 6 | 2 |
| Lng 60XL | Adenocarcinoma | Lung 2 | 1 | 4 |
| Lng AC66 | Adenocarcinoma | Lung 3 | 1 | 0 |
| Lng AC69 | Adenocarcinoma | Lung 4 | 117 | 6 |
| Lng AC88 | Adenocarcinoma | Lung 5 | 12 | 6 |
| Lng AC11 | Adenocarcinoma | Lung 6 | 1 | 18 |
| Lng AC32 | Adenocarcinoma | Lung 7 | 4 | 2 |
| Lng AC39 | Adenocarcinoma | Lung 8 | 2 | 1 |
| Lng AC90 | Adenocarcinoma | Lung 9 | 1 | 0 |
| Lng SQ9X | Squamous cell | Lung 10 | 7 | 0 |

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| | | | | |
|-----------|-----------------------------------|-----------|----|----|
| Lng SQ45 | Squamous cell carcinoma | Lung 11 | 45 | 1 |
| Lng SQ56 | Squamous cell carcinoma | Lung 12 | 1 | 23 |
| Lng SQ16 | Squamous cell carcinoma | Lung 13 | 0 | 0 |
| Lng SQ32 | Squamous cell carcinoma | Lung 14 | 9 | 5 |
| Lng SQ80 | Squamous cell carcinoma | Lung 15 | 2 | 0 |
| Lng SQ79 | Squamous cell carcinoma | Lung 16 | 5 | 11 |
| Lng C20X | Squamous cell carcinoma | Lung 17 | 0 | 0 |
| Lng 47XQ | Squamous cell carcinoma | Lung 18 | 1 | 0 |
| Lng SQ44 | Squamous cell carcinoma | Lung 19 | 1 | 0 |
| Lng BR94 | Squamous cell carcinoma | Lung 20 | 1 | 0 |
| Lng 90X | Squamous cell carcinoma | Lung 21 | 0 | 13 |
| Lng LC71 | Large cell carcinoma | Lung 22 | 31 | 12 |
| Lng LC109 | Large cell carcinoma | Lung 23 | 1 | 83 |
| Lng 75XC | Metastatic from bone cancer | Lung 24 | 2 | 4 |
| Lng MT67 | Metastatic from renal cell cancer | Lung 25 | 0 | 1 |
| Lng MT71 | Metastatic from melanoma | Lung 26 | 0 | 24 |
| Bld 32XK | | Bladder 1 | 0 | 0 |
| Bld 46XK | | Bladder 2 | 0 | 0 |
| Cln AS45 | | Colon 1 | 0 | 0 |
| Cln C9XR | | Colon 2 | 0 | 0 |

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| | | | | |
|--------------|--|---------------|-----|---|
| Cvx KS52 | | Cervix 1 | 0 | 0 |
| Cvx NK23 | | Cervix 2 | 0 | 0 |
| End 28XA | | Endometrium 1 | 7 | 0 |
| End 12XA | | Endometrium 2 | 0 | 0 |
| End 68X | | Endometrium 3 | 3 | 2 |
| End 8XA | | Endometrium 4 | 0 | 0 |
| Kid 106XD | | Kidney 1 | 0 | 0 |
| Kid 107XD | | Kidney 2 | 0 | 0 |
| Liv 94XA | | Liver 1 | 0 | 0 |
| Liv 15XA | | Liver 2 | 0 | 0 |
| Mam A06X | | Mammary 1 | 0 | 0 |
| Mam B011X | | Mammary 2 | 116 | 0 |
| Mam 47XP | | Mammary 3 | 0 | 0 |
| Mam 59X | | Mammary 4 | 1 | 0 |
| Ovr 103X | | Ovary 1 | 0 | 0 |
| Pan 71XL | | Pancreas 1 | 0 | 0 |
| Pan 77X | | Pancreas 2 | 0 | 0 |
| Pro 20XB | | Prostate 1 | 0 | 0 |
| Pro 12B | | Prostate 2 | 0 | 0 |
| SmI 21XA | | Sm. Int. 1 | 0 | 0 |
| SmI H89 | | Sm. Int. 2 | 0 | 0 |
| Sto AC44 | | Stomach 1 | 0 | 0 |
| Sto MT54 | | Stomach 2 | 0 | 0 |
| Sto TA73 | | Stomach 3 | 1 | 1 |
| Tst 39X | | Testis | 0 | 0 |
| Utr 135XO | | Uterus 1 | 0 | 0 |
| Utr 141XO | | Uterus 2 | 0 | 0 |

0= Negative

In the analysis of matching samples, the higher level of expression was in lung, showing a high degree of tissue specificity for this tissue. These results confirm the tissue specificity results obtained with normal pooled samples (Table 6).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 7 shows overexpression of LSG Lng107 (SEQ ID NO:6) in 15 lung cancer tissues compared with their respective normal adjacent (lung samples #1, 3, 4, 5, 7, 8, 9, 10, 11, 14, 15, 18, 19, 20, and 22). There is overexpression in the cancer tissue for 57% of the lung matching samples tested (total of 26 lung matching samples).

Altogether, the high level of tissue specificity, plus the mRNA overexpression in more than half of the lung matching samples tested are demonstrative of Lng107 being a diagnostic marker for lung cancer. The amino acid sequence encoded by Lng107 is depicted in SEQ ID NO:9.

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What is Claimed is:

1. A method for diagnosing the presence of lung cancer in a patient comprising:
 - (a) measuring levels of LSG in a sample of cells, tissue or bodily fluid obtained from the patient; and
 - (b) comparing the measured levels of LSG with levels of LSG in a sample of cells, tissue or bodily fluid obtained from a control, wherein an increase in measured levels of LSG in the patient versus the LSG levels in the control is associated with the presence of lung cancer.

2. A method of diagnosing metastatic lung cancer in a patient comprising:
 - (a) measuring levels of LSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and
 - (b) comparing the measured levels of LSG with levels of LSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in measured LSG levels in the patient versus the LSG levels in the control is associated with a cancer which has metastasized.

3. A method of staging lung cancer in a patient comprising:
 - (a) identifying a patient suffering from lung cancer;
 - (b) measuring levels of LSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and
 - (c) comparing the measured levels of LSG with levels of LSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in the measured levels of LSG versus the levels of LSG in the control is associated with a cancer which is progressing and a decrease in the measured levels of LSG versus the levels of LSG in the control is associated with a cancer which is regressing or in remission.

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4. A method of monitoring lung cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having lung cancer that is not known to have metastasized;

(b) periodically measuring LSG levels in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the periodically measured levels of LSG with levels of LSG in cells, tissue, or bodily fluid obtained from a control, wherein an increase in any one of the periodically measured levels of LSG in the patient versus the levels of LSG in the control is associated with a cancer which has metastasized.

5. A method of monitoring changes in a stage of lung cancer in a patient comprising:

(a) identifying a patient having lung cancer;

(b) periodically measuring levels of LSG in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of LSG with levels of LSG in a sample of the same cells, tissue, or bodily fluid of a control, wherein an increase in any one of the periodically measured levels of LSG versus levels of LSG in the control is associated with a cancer which is progressing in stage and a decrease in any one of the periodically measured levels of LSG versus the levels of LSG in the control is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4, or 5 wherein the LSG comprises SEQ ID NO: 1, 3 or 6.

SEQUENCE LISTING

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Lys Leu Ala Pro Leu Pro Leu Asp Asn Ile Leu Pro Phe Met Asp Pro
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Leu Lys Leu Leu Leu Lys Thr Leu Gly Ile Ser Val Glu His Leu Val
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Arg Val Gln Pro Gly Arg Arg Thr Leu Asn Leu Leu Arg Gly Trp Arg
 35 40 45

Glu Pro Ala Glu Leu Pro Lys Leu Gly Ala Pro Ser Pro Gly Asp Lys
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Pro Ile Phe Val Pro Leu Ser Asn Tyr Arg Asp Val Gln Tyr Phe Gly
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Glu Ile Gly Leu Gly Thr Pro Pro Gln Asn Phe Thr Val Ala Phe Asp
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Thr Gly Ser Ser Asn Leu Trp Val Pro Ser Arg Arg Cys His Phe Phe
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Ser Val Pro Cys Trp Leu His His Arg Phe Asp Pro Lys Ala Ser Ser
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Ser Phe Gln Ala Asn Gly Thr Lys Phe Ala Ile Gln Tyr Gly Thr Gly
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Lys Gly Ala Ser Val Ile Phe Gly Glu Ala Leu Trp Glu Pro Ser Leu
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Val Phe Ala Phe Ala His Phe Asp Gly Ile Leu Gly Leu Gly Phe Pro
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35 40 45

Asn Pro Leu Gly Thr Leu Asn Pro Leu Lys Leu Leu Leu Ser Ser Leu
50 55 60

Gly Ile Pro Val Asn His Leu Ile Glu Gly Ser Gln Lys Cys Val Ala
65 70 75 80

Glu Leu Gly Pro Gln Ala Val Gly Ala Val Lys Ala Leu Lys Ala Leu
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Leu Gly Ala Leu Thr Val Phe Gly
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10344

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; G01N 33/72

US CL : 435/4, 6; 436/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6; 436/64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS search terms: lung specific gene#

STN search terms: ggcaagtggaacc, ctgagagctctcaaatct, ccggcgctggaggcgagg

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X,P | WO 98/33926 A1 (ABBOTT LABORATORIES) 06 August 1998(06.08.98), see entire reference. | 1-6 |
| X | WO 98/20143 A1 (ABBOTT LABORATORIES) 14 May 1998(14.05.98), see entire reference. | 1-6 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *E* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

04 AUGUST 1999

Date of mailing of the international search report

02 SEP 1999

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